J Vet Clin 25(3) : 139-145 (2008)

Journal of Veterinary Clinics
www.ksvc.or.kr

Genotypic Diversity of the Complete Open-Reading Frame 7 Sequences of Porcine Reproductive and Respiratory Syndrome Viruses in Korea and Coexistence of Two Genotypes

Jia-Qi Chu, Myung-Cheol Kim, Chang-Sik Park*, Myung-Jo You and Moo-Hyung Jun[1]**

College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, Korea
**Research Center for Transgenic Cloned Pigs, Chungnam National University, Daejeon 305-764, Korea*
***College of Veterinary Medicine, Chonbuk National University, Jeonju 560-791, Korea*

(Accepted: June 13, 2008)

Abstract : To investigate the genotypic diversity of the porcine reproductive and respiratory syndrome viruses (PRRSV) in Korea, we examined 92 clinical samples from three provinces by RT-PCR and a nested PCR, and the complete open-reading frame 7 (ORF 7) sequences of 15 samples selected from 72 PCR-positive specimens were analyzed. When we compared nucleotide (amino acid) sequences of 80 isolates from Korea and overseas countries, the sequences of 7 samples belonged to North American (NA)-genotype, and those of 8 samples, to European (EU)-genotype. The nucleotide (amino acid) identities between two genotypes were 63.7% (59.8%) to 65.1% (63.1%). When compared with NA prototype VR-2332, the 7 strains of NA-genotype shared 89.8% (93.6%) to 91.2% (96.0%) identity of nucleotide (amino acid) sequence. The 8 strains of EU-type shared 93.6% (92.3%) to 94.3% (93.8%) identity of nucleotide (amino acid) sequence as compared to EU prototype Lelystad. In phylogenetic tree analysis by neighbor-joining method, all of the 8 EU-type strains were clustered into group 4 distinct from EU-prototype Lelystad (group 1). In NA-genotype, 24 domestic isolates reported previously and the 7 strains of NA-type determined in this study were clustered into group 1, while US prototype VR 2332 was classified into different group (group 2). These results suggest that emergence of EU-genotype and the dual-infection of NA- and EU-genotypes may be prevalent in the pig farms in Korea. The high degree of genetic diversity of field PRRSVs should be taken into consideration for control and preventive measures.

Key words **:** PRRSV, ORF7 gene, EU-genotype, Korean isolates.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically important diseases affecting the swine industry worldwide, characterized by reproductive failure in late term gestation in sows and respiratory disease in pigs of all ages (6,24). The causative agent, the PRRS virus (PRRSV), was first described as the Lelystad virus in the Netherlands (24) and VR-2332 in the U.S. (6). The two prototype virus strains share approximately 60% nucleotide identity and have been divided into two main PRRSV genotypes: European (EU; type I) and North American (NA; type II) (18).

PRRSV is a member of the family Arteriviridae and belongs to the order Nidovirales, and a small-enveloped virus with a positive-sense, single stranded RNA genome. The viral genome contains nine open reading frames (ORFs) flanked by 5' and 3' noncoding regions. ORF 1a and ORF 1b occupy more than two-thirds of the genome encoding viral replicase polyproteins. The viral structural proteins are encoded by ORFs 2-7 (17). ORF 5, 6, and 7 encode the three major structural proteins: the envelope glycoprotein (GP5), membrane (M), and nucleocapsid protein (N), respectively. ORF 2, 3, and 4 express minor membrane-associated glycoproteins designated as GP2, GP3, and GP4, respectively(17,21). N proteins along with GP5 have been demonstrated as the most immunodominant antigens in the pig immune response to PRRSV (16). ORF 7 encoding N protein is highly conserved, and the PRRSV genetic relationships by comparing the ORF 7 nucleotide sequences have been reported in different countries and regions (8,9,11,21).

The genetic diversity of PRRSV is relatively high, especially between the EU- and NA-type strains (1,13,21). Within American group of PRRSV isolates, a high degree of genetic variability has been described, while European isolates were considered to be less variable (3,18). However, recent studies showed that significant genetic heterogeneity exists within EU-type isolates (10).

Introduction of PRRSV into the Republic of Korea was dated back to as early as 1985(20). The first successful isolation of PRRSV, which was antigenically similar to contemporary U.S. PRRSV at that time, was made from the diseased

[1]Corresponding author.
E-mail : mhjun@cnu.ac.kr

pigs in 1994 (14). The subsequent genetic characterization of PRRSVs revealed that the Korean viruses belonged to NA-type (2,4,11,25). Recently, it has been reported that a Korean isolate KU-05 was classified into EU-genotype by analysis of ORF 1b sequence, and that there was not only NA-type PRRSV antibody, but also antibody to EU-type PRRSV in Korean pig farms (12).

In this study, 92 clinical samples collected from the diseased pigs in Chungnam, Gyeongbuk, and Gyeongnam provinces in 2007 were investigated by RT-PCR and nested PCR to detect ORF 7 gene of PRRSV and to differentiate the genotypes. The complete ORF 7 sequence of 15 samples detected by the nested PCR were sequenced and analyzed the genetic diversity by comparison with those of domestic and overseas isolates.

Materials and Methods

Field samples

A total of 92 clinical samples were submitted from 18 different pig herds located in Chungnam, Gyeongbuk, and Gyeongnam provinces during the period of January to December 2007. The samples consisted of 10 lungs, 57 bloods, 6 aborted fetuses, and 19 boar semen, all of which were collected from different pigs. Whole bloods were centrifuged at 3,000 rpm for 15 min to obtain serum prior to use. The lungs from diseased pigs and aborted fetuses were treated as previously described (15). Briefly, 1g of tissue was grinded together with 1 g of sterilized sea sand by using a mortar and pestle, and then suspended with 9 ml of 0.1% diethyl-pyrocarbonate (DEPC) and centrifuged at 3,000 rpm for 10 min. The supernatant fluid was collected for test. The boar semen was treated by the procedures as described by Christopher-Hennings *et al.* (5). All of the prepared samples were stored at −70°C until use.

Viral RNA extraction

Viral RNA in the serum samples (150 μl) was extracted using QIAamp® Viral RNA Mini Kit (QIAGEN, Hilden, Germany) by following the manufacturer's instructions. Total RNA was extracted from tissue suspension (150 μl) or semen cell fraction (0.5 cm^3) using RNAiso kit (TaKaRa, Japan). The extracted RNA was air-dried and dissolved in 30 μl of 0.1% DEPC-treated water prior to the reverse transcription reaction.

Oligo primers

A total of 4 paired primers for initial PCR (first-round PCR) and nested PCR (second-round PCR) were designed based on the published sequences of the EU-prototype strain Lelystad (accession no. M 96262) and/or NA- prototype VR-2332 (accession no. U87392) using software PrimerPremier (Version 5.0) and reconfirmed by software Oligo (Version 6.0). The primers for initial RT-PCR were designed to generate the ORF 7 gene together with parts of ORF 6 and 3' end, and the nested primers for second-round amplification, the whole ORF 7 gene only. The primers synthesized by GENOTECH CO. (Korea) were listed in Table 1.

Initial RT-PCR and nested PCR

Reverse transcription reaction was performed in a volume of 20 μl containing 10 μl RNA, 4 μl Reverse Transcriptase Premix (Elpis Biotech, Korea) and 6 μl 0.1% DEPC-treated water, followed by incubating at 42°C for 60 min and 94°C for 5 min. Five μl of the reverse transcription product was used as template in the initial PCR. The amplification was carried out in a final volume of 50 μl containing 10x Ex *Taq* buffer (20 mM Mg^{2+}), dNTPs (2.5 mM each), 20 pmol of sense and antisense primer, 1.25 U Ex *Taq* Polymerase (TaKaRa, Japan). The first-round PCR cycle conditions were as follows: 94°C for 5 min and 30 cycles of 94°C for 1 min, 58°C for 1 min and 72°C for 1 min, with a final prolongation for 5 min at 72°C One μl of first-round PCR product was used as template of the nested PCR. Conditions for nested PCR were almost the same as the first-round PCR. Ten μl of nested PCR product was mixed with 1 μl of 10x loading buffer (TaKaRa, Japan) and loaded on a 1.5% agarose gel,

Table 1. Primers used in PCR for ORF 7 gene and the size of the expected amplified products

Genotype	PCR step	S/AS*	Sequence (5'-3')	Genome location (nt)**	Product size(bp)
European (EU)	Initial	S	GAAAGCCCGGACTAACATCA	14490-14509	546
		AS	AGGTGACTCAGAGGCCACAC	15016-15035	
	Nested	S	GGCAAACGAGCTGTTAAACG	14555-14574	451
		AS	GCCATTCACCTGACTGTCAAA	14985-15005	
North American (NA)	Initial	S	ACTACGGTCAACGGCACATT	14795-14814	526
		AS	TGCCATTCACCACACATTCT	15301-15320	
	Nested	S	CGGGTTAAAAAGCCTCGTGT	14821-14840	474
		AS	TCAAACACTGAGATGCCTCAA	15274-15294	

*S: the sense primer. AS: anti-sense primer

**Nucleotide location on the PRRSV genome was given in reference to the sequences of European strain Lelystad (GenBank accession no. M96262) and North American strain VR-2332 (GenBank accession no. U87392).

Table 2. Origin of PRRSV ORF 7 sequences investigated in this study

Strain/ Sample	Genotype	Origin	Yr/Mo	GenBank accession no.	Strain/ Sample	Genotype	Origin	Yr / Mo	GenBank accession no.
21191	EU	Denmark	1997	AY035953	05K170	NA	Korea (Gyeonggi)	2005	EF441807
12654	EU	Denmark	1995	AY035947	06K648	NA	Korea (Gyeonggi)	2006	EF441844
5767-6	EU	Denmark	1995	AY035962	06K672	NA	Korea (Gyeonggi)	2006	EF441848
IV/92	EU	France	1992	Z92528	06K919	NA	Korea (Gyeonggi)	2006	EF441858
SDRPV4A	EU	France	1993	AY035965	03KSJY32	NA	Korea (Gangwon)	2003	EF441782
2.25	EU	Germany	1993	Z92538	05K168	NA	Korea (Gangwon)	2005	EF441806
2.46	EU	Germany	1993	AY035967	06K017	NA	Korea (Jeonbuk)	2006	EF441838
2156	EU	Italy		AY035974	03KKKB37	NA	Korea (Chungnam)	2003	EF441785
1828	EU	Italy		AY035971	05K267	NA	Korea (Chungnam)	2005	EF441829
Lelystad	EU	The Netherlands	1991	M96262	05K263	NA	Korea (Chungbuk)	2005	EF441827
LV4.2.1	EU	The Netherlands		AY588319	05K234	NA	Korea (Gyeonggi)	2005	EF441815
NL2.2	EU	The Netherlands	1991	Z92533	05K220	NA	Korea (Jeonnam)	2005	EF441812
L51/2/92	EU	Spain		Z92531	05K116	NA	Korea (Jeonnam)	2005	EF441793
H2	EU	Great Britain	1991	AF378799	06K015	NA	Korea (Chungnam)	2006	EF441837
NY3-D769	EU	UK	1992	Z92536	05K123	NA	Korea (Gyeongnam)	2005	EF441796
L2	EU	UK	1992	L77919	MD-001	NA	Taiwan	1991	AF121131
Ox1	EU	UK		L77927	SIN	NA	Singapore	1999	AF184212
H2-D768	EU	UK	1991	AY035981	Aus	NA	Lithuania	2000	AF378800
Nie	EU	Poland	1997	AF438361	Sid	NA	Lithuania	2000	AF378801
Upa-13	EU	Poland		DQ324721	VR-2332	NA	USA		U87392
Sok-9	EU	Poland		DQ324716	ISU-P	NA	USA		AF043974
Rak	EU	Poland	1997	AF438360	ISU-55	NA	USA		U18751
EuroPRRSV	EU	USA		AY366525	92-11824	NA	USA	1992	AF043950
SDPRRS 02-11	EU	USA		AY749400	22aD	NA	Mexico		AY209217
SD01-08	EU	USA		DQ489311	SP	NA	Vaccine strain (Prime Pac)		AF184212
MN-03-01_EU	EU	USA		AY749378	**PicSer1V***	**NA**	**Korea (Gyeongbuk)**	**2007/12**	**EU424157**
N-34	EU	China		EF537859	**PicSer2V**	**NA**	**Korea (Gyeongbuk)**	**2007/12**	**EU424158**
Ningbo57	EU	China		EF473139	**PicSer2L**	**EU**	**Korea (Gyeongbuk)**	**2007/12**	**EU424159**
v1	EU	Modified live vaccine		DQ009649	**PicLun5L**	**EU**	**Korea (Gyeongnam)**	**2007/10**	**EU424160**
AGS-96	EU	Austria		AF512378	**PicSer13L**	**EU**	**Korea (Gyeongbuk)**	**2007/12**	**EU424161**
92V58	EU	Belgium	1992	U40701	**PicAbortSer8L**	**EU**	**Korea (Gyeongbuk)**	**2007/12**	**EU424162**
AV30	EU	Belgium	1992	U40700	**DsSem4V**	**NA**	**Korea (Chungnam)**	**2007/08**	**EU424163**
PA8	NA	Canada	1995	AF176348	**DsSer8V**	**NA**	**Korea (Chungnam)**	**2007/09**	**EU424164**
IAF-exp91	NA	Canada		L40898	**DsSer9V**	**NA**	**Korea (Chungnam)**	**2007/09**	**EU424165**
BJ-4	NA	China		AF331831	**DsSem4L**	**EU**	**Korea (Chungnam)**	**2007/08**	**EU424166**
CH-1a	NA	China		AY032626	**DsSem6L**	**EU**	**Korea (Chungnam)**	**2007/08**	**EU424167**
Kitasato 93-1	NA	Japan		AB023782	**DsAbort1V**	**NA**	**Korea (Chungnam)**	**2007/03**	**EU424168**
PL97-1	NA	Korea	1997	AY585241	**DsAbort3V**	**NA**	**Korea (Chungnam)**	**2007/03**	**EU424169**
06K915	NA	Korea (Gyeongbuk)	2006	EF441856	**DsAbort3L**	**EU**	**Korea (Chungnam)**	**2007/03**	**EU424170**
06K1083	NA	Korea (Gyeonggi)	2006	EF441865	**DsAbort12L**	**EU**	**Korea (Chungnam)**	**2007/12**	**EU424171**

* Fifteen samples investigated in this study are indicated in boldface type.

stained with ethidium bromide and visualized by Image-analyzer (Pharmacia Biotech, USA). The positive bands at 474 bp and 451 bp were determined as NA-type and EU-type, respectively. The specificity of PCR was confirmed with the virus in Ingelvac® PRRS MLV (Modified live PRRS virus, Berlinger Ingelheim, USA).

Sequencing and phylogenetic analysis

The PCR product for direct sequencing was purified in 1.5% (w/v) agarose gel and then extracted with Gel extraction kit (GENEALL, Korea) by following the manufacturer's instructions. The purified products were sent to COSMO CO. (Korea) for nucleotide sequencing. The sequences were assembled using SeqMan (Lasergene program package, DNASTAR Inc., Madison, WI, USA). Multiple sequence alignment for the complete ORF 7 sequences (372bp for NA-type and 387bp for EU-type) was carried out using the MegAlign (Lasergene package, DNASTAR Inc., USA). The graphical output of the phylogenetic tree were generated by the distance-based neighbor-joining method using MEGA software (Version 3.1). The deduced amino acid sequences were aligned using software ClustalX 1.83 to investigate the divergence of amino acid sequences of ORF 7 sequences of PRRSVs listed in Table 2 (23). The sequences determined from this study were deposited in the GenBank under the accession no. EU424157 to EU424171 (Table 2).

Results

Detection and differentiation of viral RNA

Of 92 clinical samples investigated by initial RT-PCR and nested PCR, 72 (78.3%) samples were positive for ORF 7 gene. Of 72 positive-samples, 29 (31.5%) samples showed the positive band as EU-type (451 bp), and 8 (8.7%) samples, as NA-type (474 bp), and the remaining 35 (38.0%) samples were dually infected with EU- and NA- types. NA-type PRRSV and dual-infection were distributed in all organs tested, while EU-type PRRSV was detected in the samples of lung, blood, and boar semen (Table 3). Fifteen PCR products consisted of 8 EU-types and 7 NA-types were selected for sequencing and genetic analysis (Table 2).

Nucleotide sequencing and analysis

The nucleotide and amino acid sequences of the complete ORF 7 gene of 15 strains determined in this study and those of EU- and NA- prototypes were compared in pairs (Table 4). Seven strains (PicSer1V, PicSer2V, DsSem4V, DsSer8V, DsSer9V, DsAbort1V, and DsAbort3V) detected in this study shared 89.8% (93.6%) to 91.2% (96.0%) identity of nucleotide (amino acid) sequence as compared with NA prototype VR-2332. Eight strains (PicSer2L, PicLun5L, PicSer13L, PicAbortSer8L, DsSem4L, DsSem6L, DsAbort3L, DsAbort 12L) detected in this study shared 93.6% (92.3%) to 94.3% (93.8%) identity of nucleotide (amino acid) sequence as compared with EU prototype Lelystad. When we compared the nucleotide (amino acid) sequences of the 8 strains of NA-type with those of the 9 strains of EU-type, the identities were 63.7% (59.8%) to 65.9% (63.9%) (Table 4). In comparison of the nucleotide (amino acid) sequences among the 7 strains of NA-type, 92.5% (96.0%) to 100% (100%) identities were observed, while the nucleotide (amino acid) sequences among the 8 strains of EU-type showed 97.7% (95.4%) to 100% (100%) identities (Table 4).

Phylogenetic analysis

Phylogenetic analysis based on the complete ORF 7 sequences were conducted for 40 NA-genotype PRRSVs comprised of 25 isolates from Korea and 15 isolates from overseas countries, and 40 EU-genotype PRRSVs including the 8 strains detected in this study and 32 isolates from overseas countries. In analysis of genetic relationship among EU-genotypes (Fig 1A), EU prototype Lelystad was classified into group 1, while all of the 8 strains of EU-type were clustered into group 4 together with two Polish isolates. In analysis of 40 NA genotypes PRRSVs (Fig 1B), 24 domestic isolates including the 7 strains were clustered into group 1 together with a Taiwanese isolate. Within group 1, 4 strains detected in this study formed a clade with a closer relationship with the majority of Korean iso-

Table 3. Detection of EU- and NA- genotypes from various specimens collected from the pig farms during 2007 by nested PCR

Specimens (No.)	No. of samples positive (%)			No. of samples negative (%)
	EU-type	NA-type	Dual-infection*	
Lung (10)	2 (20.0)	1 (10.0)	2 (20.0)	5 (50.0)
Blood (57)	21 (36.8)	3 (5.3)	26 (45.6)	7 (12.3)
Aborted fetus (6)	0 (0)	2 (33.3)	2 (33.3)	2 (33.3)
Boar semen (19)	6 (31.6)	2 (10.5)	5 (26.3)	6 (31.6)
Total (92)	29 (31.5)	8 (8.7)	35 (38.0)	20 (21.7)

*The samples showed simultaneously the positive bands for both of EU- and NA-types.

Table 4. Pairwise comparison of the nucleotide and amino acid sequences of the complete ORF 7 gene of fifteen isolates detected in this study and the EU- and NA- prototype PRRSV

		Percentages of amino acid sequence identity																
NO.	Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	VR-2332	*	93.6	93.6	94.4	96.0	96.0	94.4	94.4	62.3	62.3	62.3	63.1	63.1	63.9	63.1	63.1	62.3
2	DsAbort3V	90.1	*	98.4	99.2	96.0	96.0	99.2	99.2	59.8	59.8	59.8	60.7	60.7	61.5	60.7	60.7	59.8
3	DsAbort1V	89.8	99.2	*	99.2	96.0	96.0	99.2	99.2	60.7	60.7	60.7	61.5	61.5	62.3	61.5	61.5	60.7
4	DsSem4V	90.3	99.7	99.5	*	96.8	96.8	100.0	100.0	60.7	60.7	60.7	61.5	61.5	62.3	61.5	61.5	60.7
5	DsSer8V	91.2	92.5	92.8	92.8	*	100.0	96.8	96.8	62.3	62.3	62.3	63.1	63.1	63.9	63.1	63.1	62.3
6	DsSer9V	91.2	92.5	92.8	92.8	100.0	*	96.8	96.8	62.3	62.3	62.3	63.1	63.1	63.9	63.1	63.1	62.3
7	PicSer1V	90.9	99.2	98.9	99.5	93.3	93.3	*	100.0	60.7	60.7	60.7	61.5	61.5	62.3	61.5	61.5	60.7
8	PicSer2V	90.3	99.7	99.5	100.0	92.8	92.8	99.5	*	60.7	60.7	60.7	61.5	61.5	62.3	61.5	61.5	60.7
9	PicSer13L	64.6	64.0	64.0	64.3	64.8	64.8	64.3	64.3	*	96.2	96.2	97.7	95.4	92.3	96.9	96.9	96.2
10	DsAbort3L	64.3	63.7	63.7	64.0	64.6	64.6	64.0	64.0	97.9	*	100.0	96.9	99.2	93.1	97.7	97.7	100.0
11	DsAbort12L	64.3	63.7	63.7	64.0	64.6	64.6	64.0	64.0	97.9	100.0	*	96.9	99.2	93.1	97.7	97.7	100.0
12	DsSem4L	65.1	64.6	64.6	64.8	64.8	64.8	64.8	64.8	98.2	98.7	98.7	*	97.7	93.1	99.2	99.2	96.9
13	DsSem6L	64.6	64.0	64.0	64.3	64.8	64.8	64.3	64.3	97.7	99.7	99.7	99.0	*	93.8	98.5	98.5	99.2
14	Lelystad	65.9	64.3	64.3	64.6	65.7	65.7	64.6	64.6	93.8	93.8	93.8	93.6	94.1	*	93.8	93.8	93.1
15	PicAbortSer8L	65.4	64.8	64.8	65.1	65.1	65.1	65.1	65.1	98.5	98.5	98.5	99.2	98.7	94.3	*	98.5	97.7
16	PicLun5L	64.6	64.3	64.3	64.6	65.1	65.1	64.6	64.6	98.2	98.2	98.2	98.5	98.5	94.3	98.7	*	97.7
17	PicSer2L	64.3	63.7	63.7	64.0	64.6	64.6	64.0	64.0	97.9	100.0	100.0	98.7	99.7	93.8	98.5	98.2	*

Percentages of nucleotide sequence identity

lates reported previously. Within group 1, 2 strains with 100% identity, DsSer8V and DsSer9V, detected in this study, formed a clad apart from the other 5 strains. A Korean isolate, PL97-1 reported in 1997, belonged to a same cluster (group 2) together with U.S. prototype VR 2332.

Discussion

In the present study, we designed a nested PCR to detect ORF 7 gene of PRRSV and to differentiate between two genotypes. In the PCR, relatively high positive-rates (78.3%) of 92 samples were observed (Table 3). This result may be due to sampling from the pig farms highly endemic with PRRS and from the herds vaccinated routinely with NA-type modified-live PRRS vaccine. The high incidence of positive cases (68.4%) of boar semen implicates that boar semen may play a role of PRRSV transmission to sows in Korean pig farms. It has been reported that the boar experimentally infected with PRRSV disseminated the virus for a longer period regardless of the host genetic factors (5).

Since the first isolation of PRRSV from the diseased pigs in Korea (14), it has been recognized that the major genotypes of PRRSVs circulating in swine farms are NA-type with some extent of genetic variation (2,11,25). Recently, the emergence of EU-type strain has been reported by genetic analysis of ORF 1b region of a Korean isolate KU-05 and by serological test (12). The present data based on the complete ORF 7 sequence provide more evidences that EU-genotype PRRSVs have been widely circulated in the pig farms in Korea, and that dual-infection of NA- and EU-genotypes are common in the clinical cases of PRRS. Recently, intermingling infection of the two genotypes have been reported in Europe, Asia as well as North America leading to significant impacts on diagnostics, control, and farm management for PRRS (7,10,19,22). The significance of co-exist of two genotypes and emergence of EU-genotype in Korean pig farms should be studied further for PRRS control and prevention.

When we compared the complete ORF 7 sequences of 80 isolates reported from Korea and overseas countries. Of 15 strains detected in this study, ORF 7 sequences of 7 samples

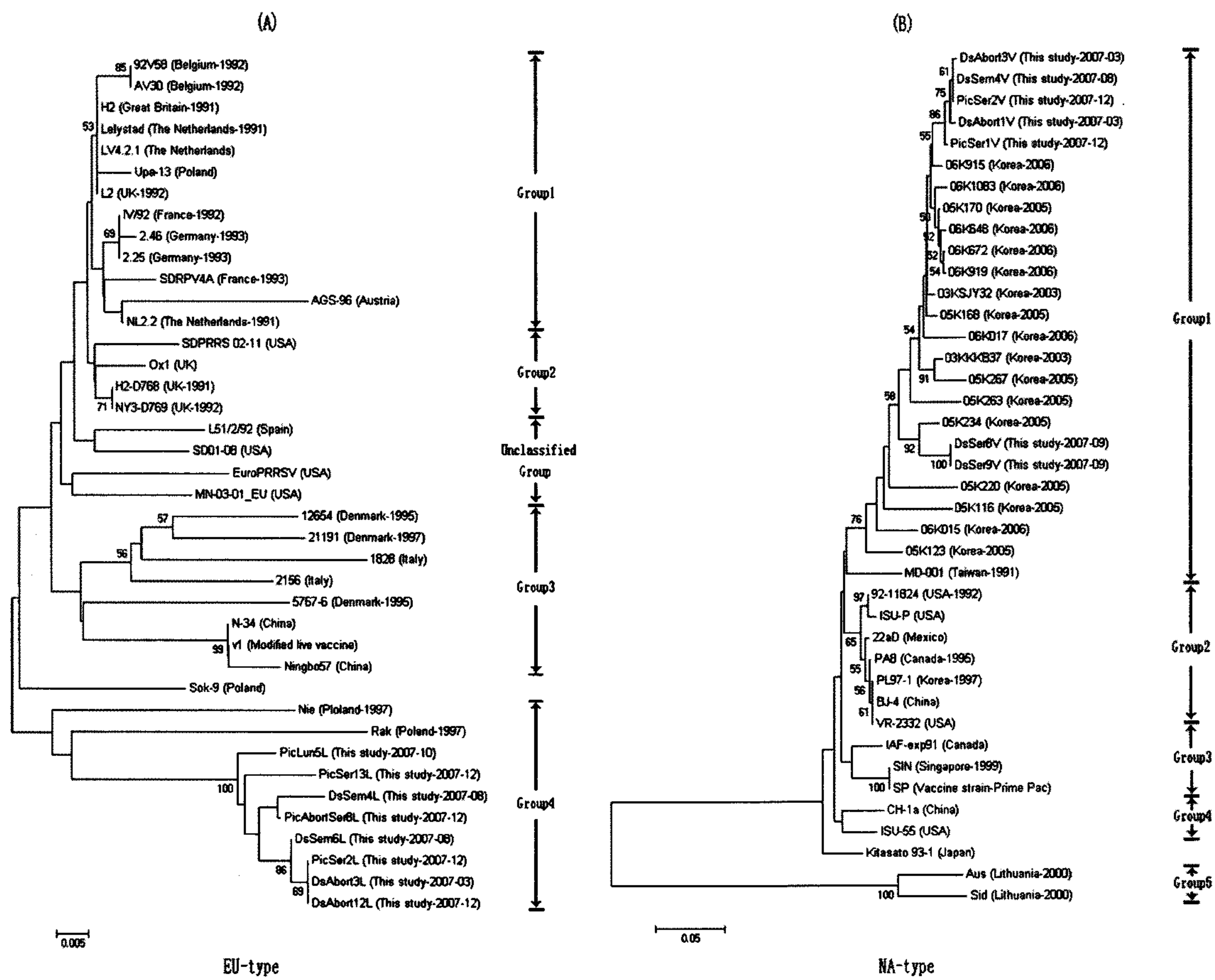

Fig 1. Phylogenetic relationships of 15 complete ORF 7 sequences determined in this study and 65 selected PRRSVs from overseas countries. The phylogenetic trees for EU-type (A) and NA-type (B) were constructed by neighbor-joining method. Bootstrap values equal or over than 50% (1,000 replicates) were indicated on the branches.

were classified into NA-type, and those of 8 samples, into EU-type PRRSV. It has been known that the genetic variation of EU-genotypes were less variable than that of NA genotypes (1,3,13,18,21). In our study, the similar results were obtained since the sequences of the 7 strains of NA-type revealed the wider range of variation in nucleotide sequences (homology range of 89.8% to 91.2%) than those of the 8 EU-type strains (homology range of 93.6% to 94.3%).

The phylogenetic tree analysis shows that both genotypes of Korean isolates are clustered into a distinct subgroup apart from each of prototype strains, and that a clade is distinct from the non-Korean PRRSVs. Overall, it is clear that the sequences of ORF 7 gene of NA-type strains circulating in the domestic pig farms are continually changing by year of isolation, leading to a distinct group of virus (25). Interestingly, among the Korean NA-type isolates, only PL97-1 isolate is located in the site nearby VR-2332 in group 2. This might be related with the suggestion that PL97-1 may be reverted from the VR-2332-derived vaccine strain (11).

In conclusion, the genetic varieties among PRRSVs in Korea need to take into consideration for the current strategy for diagnosis, prevention and control of PRRS.

Acknowledgments

This work was supported by grant No. R11-2002-100-00000-0 from ERC program of the Korea Science & Engineering Foundation. The authors would like to thank Ha-yoon Lee for her technical assistance.

References

1. An TQ, Zhou YJ, Liu GQ, Tian ZJ, Li J, Qiu HJ, Tong GZ. Genetic diversity and phylogenetic analysis of glycoprotein 5 of PRRSV isolates in mainland China from 1996 to 2006: Coexistence of two NA-subgenotypes with great diversity. Veterinary Microbiology 2007: 123; 43-52.
2. Cha SH, Choi EJ, Park JH, Yoon SR, Song JY, Kwon JH, Song HJ, Yoon KJ. Molecular characterization of recent Korean porcine reproductive and respiratory syndrome (PRRS) viruses and comparison to other Asian PRRS viruses. Veterinary Microbiology 2006: 117; 248-257.
3. Chang CC, Yoon KJ, Zimmerman JJ, Harmon KM, Dixon PM, Dvorak CMT, Murtaugh MP. Evolution of porcine reproductive and respiratory syndrome virus during sequential passages in pigs. J Virol 2002: 76; 4750-4763.

4. Cheon DS, Chae CH. Restriction fragment length polymorphism analysis of open reading frame 5 gene of porcine reproductive and respiratory syndrome virus isolates in Korea. Arch Virol 2000: 145; 1481-1488.
5. Christopher-Hennings J, Holler LD, Benfield DA, Nelson EA. Detection and duration of porcine reproductive and respiratory syndrome virus in semen, serum, peripheral blood mononuclear cells, and tissues from Yorkshire, Hampshire, and Landrace boars. J Vet Diagn Invest 2001: 13; 133-142.
6. Collins JE, Benfield DA, Christianson WT, Harris L, Hennings JC, Shaw DP, Goyal SM, McCullough S, Morrison RB, Joo HS, Gorcyca D, Chladek D. Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) from the disease in gnotobiotic pigs. J Vet Diagn Inves 1992: 4; 117-126.
7. Dewey C, Charbonneau G, Carman S, Hamel A, Nayar G, Friendship R, Eernisse K, Swenson S. Lelystad-like strain of porcine reproductive and respiratory syndrome virus (PRRSV) identified in Canadian swine. Can Vet J 2000: 21; 493–494.
8. Forsberg R, Storgaard T, Nielsen HS, Oleksiewicz MB, Cordioli P, Sala G, Hein J, Bôtner A. The genetic diversity of European type PRRSV is similar to that of the North American type but is geographically skewed within Europe. Virology 2002: 299; 38-47.
9. Guarino H, Goyal SM, Murtaugh MP, Morrison RB, Kapur V. Detection of porcine reproductive and respiratory syndrome virus by reverse transcription–polymerase chain reaction using different regions of the viral genome. J Vet Diagn Invest 1999: 11; 27-33.
10. Indik S, Schmoll F, Sipos W, Klein D. Genetic variability of PRRS virus in Austria: consequences for molecular diagnostics and viral quantification. Veterinary Microbiology 2005: 107; 171-179.
11. Kang SY, Yun SI, Park HS, Park CK, Choi HS, Lee YM. Molecular characterization of PL97-1, the first Korean isolate of the porcine reproductive and respiratory syndrome virus. Virus Res 2004: 104; 165-179.
12. Kim JY, Lee SY, Sur JH, Lyoo YS. Serological and genetic characterization of the European strain of the porcine reproductive and respiratory syndrome virus isolated in Korea. Korean J Vet Res 2006: 46; 363-370.
13. Kiss I, Sámi L, Kecskeméti S, Hanada K. Genetic variation of the prevailing porcine respiratory and reproductive syndrome viruses occurring on a pig farm upon vaccination. Arch Virol 2006: 151; 2269-2276.
14. Kweon CH, Kwon BJ, Lee HJ, Cho JJ, Hwang EK, Shin JH, Yoon YD, Kang YB, An SH, Kim YH, Huh W, Jun MH, Wensvoort G. Isolation of porcine reproductive and respiratory syndrome virus (PRRS virus) in Korea. Korean J Vet Res 1994: 34; 77-83.
15. Mengeling WL, Larger KM, Vorwald AC. Diagnosis of porcine reproductive and respiratory syndrome. J Vet Diagn Invest 1995: 7; 3-16.
16. Meulenberg JJM, Bende RJ, Pol JMA, Wensvoort G, Moorman RJM. Nucleocapsid protein of Lelystad virus. Expression by recombinant baculovirus : immunological properties and suitability for the detection of serum antibodies. Clin Diagn Lab Immunol 1995: 2; 652-656.
17. Meulenberg JJ, Petersen-Den Besten A, de Kluyer EP, Moormann RJ, Schaaper WM, Wensvoort G. Characterization of proteins encoded by ORFs 2 to 7 of Lelystad virus. Virology 1995: 206; 155–163.
18. Nelsen CJ, Murtaugh MP, Faaberg KS. Porcine reproductive and respiratory syndrome virus comparison: Divergent evolution on two continents. J Virol 1999: 73; 270-280.
19. Ropp SL, Mahlum Wees CE, Fang Y, Nelson EA, Rossow KD, Bien M, Arndt B, Preszler S, Steen P, Christopher-Hennings J, Collins JE, Benfield DA, Faaberg KS. Characterization of emerging European-like porcine reproductive and respiratory syndrome virus isolates in the United States. J Virol 2004: 78; 3684-3703
20. Shin JH, Kang YB, Kim YJ, Yeom SH, Kweon CH, Lee WY, Jean YH, Hwang EK, Rhee JC, An SH, Cho IS, Oh JS, Joo HS, Choi CS, Molitar TW. Seroepidemiological studies on porcine reproductive and respiratory syndrome in Korea. Part I. Detection and indirect fluorescent antibodies. RDA J Agri Sci 1993: 35; 572-576.
21. Stadejek T, Oleksiewicz MB, Potapchuk D, Podgórska K. Porcine reproductive and respiratory syndrome virus strains of exceptional diversity in eastern Europe support the definition of new genetic subtypes. J Gen Virol 2006: 87; 1835-1841.
22. Thanawongnuwech R, Amonsin A, Tatsanakit A, Damrongwatanapokin. Genetics and geographical variation of porcine reproductive and respiratory syndrome virus (PRRSV) in Thailand. Veterinary Microbiology 2004; 101; 9-21.
23. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 1997: 25; 4876-4882.
24. Wensvoort G, Terpstra C, Pol JM, Ter Laak EA, Bloemraad M, De Kluyver EP, Kragten C, Van Buiten L, Den Besten A, Wagenaar F, Broekhuijsen JM, Moonen PLJM, Zetstra T, De Boer EA, Tibben HJ, De Jong MF, Vant Veld P, Groenland GJR, Van Gennep JA, Voets MT, Verheijden JHM, Braamskamp J. Mystery swine disease in the Netherlands: the isolation of Lelystad virus. Vet Q 1991: 13; 121-130.
25. Yoon SH, Song JY, Lee CH, Choi EJ, Choi IS, Kim B. Genetic characterization of the Korean porcine reproductive and respiratory syndrome viruses based on the nucleocapsid protein gene (ORF7) sequences. Arch Virol 2008: 153; 627-635.